

Applicant : Short, et al.
Serial No. : 09/997,807
Filed : November 30, 2001
Page : 2 of 16

Attorney's Docket No.: 09010-910001

RECEIVED
CENTRAL FAX CENTER

OCT 29 2003

Amendment to the Claims:

Please cancel claims 1 to 30, 38 to 40, 43 to 113, and 116 to 131, without prejudice.

Please amend the claims as follows:

This listing of claims will replace all prior versions, and listing, of claims in the application:

Listing of Claims:

Claims 1 to 30 (canceled)

Claim 31 (currently amended) A method of producing a polypeptide polymer [[by self-assembly]] comprising the steps of:

(a) providing a plurality of monomeric polypeptides and at least one divalent cation, wherein the monomer polypeptides are capable of self-assembly in the presence of a divalent cation; and

(b) (i) polymerizing the monomeric polypeptides through a self-assembly process in the presence of [[a]] at least one divalent cation, or, (ii) polymerizing the monomeric polypeptides in the presence of [[and]] a template molecule.

Claim 32 (currently amended): The [[A]] method [[as claimed in]] of claim 31, wherein the monomeric polypeptide has [[a]] an amino acid sequence as set forth in SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, or SEQ ID NO:10 selected from the group consisting of [[:]]

~~(a) an amino acid sequence selected from SEQ ID NOS: 2, 4, 6, 8 and 10;~~

~~(b) an amino acid sequence selected from the group consisting of SEQ ID NOS: 2, 4, 6, 8 and 10, wherein the amino acid sequence has at least one conservative substitution [[; wherein]] and the polypeptide comprising the amino acid sequence (b) can self-assemble to form a polymer; and~~

~~(c) an amino acid sequence comprising a fragment of at least one amino acid residue of SEQ ID NOS: 2, 4, 6, 8 and 10, wherein the polypeptide comprising the amino acid sequence (c) can self-assemble to form a polymer.~~

OFFICIAL

Applicant : Short, et al.
Serial No. : 09/997,807
Filed : November 30, 2001
Page : 3 of 16

Attorney's Docket No.: 09010-910001

Claim 33 (currently amended): The ~~[[A]]~~ method ~~[[as claimed in]]~~ of claim 31, wherein the polypeptide is encoded by a nucleic acid ~~molecule selected from the group consisting of~~

~~(a) a nucleic acid molecule comprising a sequence having at least about 50% sequence identity [[homology]] with [[the] a nucleic acid sequence as set forth in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, or SEQ ID NO:9 of at least one of SEQ ID NOS: 1, 3, 5, 7 and 9, over a subsequence of at least about 100 residues;~~

~~(b) a nucleic acid molecule which hybridizes under low, moderate or high stringency conditions with at least one of (i) the nucleic acid sequences of SEQ ID NOS: 1, 3, 5, 7 and 9, (ii) a complementary strand of a nucleic acid sequence of at least one of SEQ ID NOS: 1, 3, 5, 7 and 9, and a subsequence of at least about 100 residues;~~

~~(c) a subsequence of (a) or (b), wherein the subsequence encodes a polypeptide, which can self assemble to form a polymer; and~~

~~(d) a nucleic acid molecule that encodes a polypeptide having an amino acid sequence that has at least about 50% identity with at least one amino acid sequence of SEQ ID NOS: 1, 3, 5, 7 and 9, as determined by analysis with a sequence comparison algorithm or by visual inspection.~~

Claim 34 (currently amended): The method ~~[[as claimed in]]~~ of claim 31, wherein the step of providing a plurality of polypeptides further comprises the steps of:

preparing a vector comprising ~~[[with]]~~ a nucleic acid ~~[[attached]]~~, wherein the nucleic acid encodes the polypeptide;

inserting the vector into a host cell;

growing the host cell in a suitable culture to express the nucleic acid to form the polypeptide; and

isolating the formed polypeptide from the host cell.

Claim 35 (currently amended): The method ~~[[as claimed in]]~~ of claim 31, wherein the step of polymerizing the polypeptides further comprises the steps of:

Applicant : Short, et al.
Serial No. : 09/997,807
Filed : November 30, 2001
Page : 4 of 16

Attorney's Docket No.: 09010-910001

dissolving the plurality of polypeptides in a solution; and
adding a template molecule and an alkaline earth metal ion [[ions]] to the
solution.

Claim 36 (currently amended): The method [[as claimed in]] of claim 34,
wherein the vector comprises plasmid pEX-CAN-A.

Claim 37 (currently amended): The method [[as claimed in]] of claim 36,
wherein the host cell comprises a host cell selected from the group consisting of an *E. coli* [[E.
Coli BL21]] (DE3) and a *Pseudomonas* [[pseudomonas]].

Claims 38 to 40 (canceled)

Claim 41 (currently amended): A method of encapsulating a molecule
comprising the steps of:

providing a solution of a plurality of polypeptides having [[a]] an amino acid
sequence selected from the group consisting of as set forth in SEQ ID NO:2, SEQ ID NO:4, SEQ
ID NO:6, SEQ ID NO:8, or SEQ ID NO:10 [[:]

(a) an amino acid sequence selected from SEQ ID NOS: 2, 4, 6, 8 and 10;

(b) an amino acid sequence selected from the group consisting of SEQ ID NOS:
2, 4, 6, 8 and 10, wherein the amino acid sequence has at least one conservative substitution [[;
wherein]] and the polypeptides polypeptide comprising the amino acid sequence (b) can self-
assemble to form a polymer; and

(c) an amino acid sequence comprising a fragment of at least one amino acid
residue of SEQ ID NOS: 2, 4, 6, 8 and 10; wherein the polypeptide comprising the amino acid
sequence (c) can self assemble to form a polymer and

polymerizing the plurality of polypeptides the presence of the molecule so as to
encapsulate the molecule in the polymer.

Applicant : Short, et al.
Serial No. : 09/997,807
Filed : November 30, 2001
Page : 5 of 16

Attorney's Docket No.: 09010-910001

Claim 42 (currently amended): The method [[as claimed in]] of claim 41, wherein at least one of said polypeptides comprises a [[target vector]] a sequence as set forth in SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, or SEQ ID NO:10.

Claim 43 to 113 (canceled)

Claim 114 (currently amended): The method [[as claimed in]] of claim 34, wherein the vector is selected from the group consisting of viral vectors, plasmid vectors, phage vectors, phagemid vectors, cosmids, fosmids, bacteriophages, artificial chromosomes, adenovirus vectors, retroviral vectors, and adeno-associated vectors.

Claim 115 (currently amended): The method [[as claimed in]] of claim 34, wherein the host is selected from the group consisting of prokaryotes, eukaryotes, fungi, yeasts, plants and metabolically rich hosts.

Claims 116 to 131 (canceled)

Claim 132 (new): The method of claim 31, wherein the monomeric polypeptides have a molecular weight of more than 5,000 daltons.

Claim 133 (new): The method of claim 132, wherein the monomeric polypeptides have a molecular weight of more than 10,000 daltons.

Claim 134 (new): The method of claim 31, wherein the monomeric polypeptides polymerize to form a hollow tube, a tubule, a micelle or a molecular sieve.

Claim 135 (new): The method of claim 134, wherein the hollow tube has approximately a 25 nm outer diameter and a 20 nm inner diameter.

Applicant : Sh rt, et al.
Serial No. : 09/997,807
Filed : November 30, 2001
Page : 6 of 16

Attorney's Docket No.: 09010-910001

Claim 136 (new): The method of claim 31, wherein the monomeric polypeptides are polymerized in the presence of a divalent cation and a template molecule.

Claim 137 (new): The method of claim 31, wherein the template molecule comprises a plasmid, a phage, a cosmid, a phagemid, a virus or a portion of a virus.

Claim 138 (new): The method of claim 137, wherein the virus comprises a retrovirus, a parainfluenzavirus, a herpesvirus, a reovirus or a paramyxovirus.

Claim 139 (new): The method of claim 137, wherein the portion of a virus comprises a coat protein, a spike glycoprotein or a capsid protein.

Claim 140 (new): The method of claim 31, wherein the plurality of monomeric polypeptides are polymerized in the presence of at least one divalent cation selected from the group consisting of Ca^{2+} , Mg^{2+} , Cu^{2+} , Zn^{2+} , Sr^{2+} , Ni^{2+} , Mn^{2+} and Fe^{2+} .

Claim 141 (new): The method of claim 31, wherein the plurality of monomeric polypeptides are polymerized in the presence of Ca^{2+} and Mg^{2+} .

Claim 142 (new): The method of claim 31, wherein the step of polymerizing the monomeric polypeptides further comprises the step of dissolving the monomeric polypeptides in an aqueous solution.

Claim 143 (new): The method of claim 31, wherein the template molecule is prepared by fragmenting or shearing of a suspension of a polymer.

Claim 144 (new): The method of claim 31, wherein the monomeric polypeptides or polymers interact with each other by pairing, bundling, entangling or electrostatic cross-linking, thereby generating paired polymers, bundled polymers, entangled polymers, cross-linked polymers or an interconnected network of polymers.

Applicant : Short, et al.
Serial No. : 09/997,807
Filed : November 30, 2001
Page : 7 of 16

Attorney's Docket N .: 09010-910001

Claim 145 (new): The method of claim 31, further comprising providing a therapeutic agent or a drug molecule and adding the therapeutic agent or drug molecule to the polymerization step, thereby generating a therapeutic agent or drug molecule encapsulated by the polymers.

Claim 146 (new): The method of claim 145, wherein the therapeutic agent or drug molecule is added to the polymerization step when a partially formed polymer is formed.

Claim 147 (new): The method of claim 146, further comprising capping the partially formed polymer using a capping unit.

Claim 148 (new): The method of claim 147, wherein the capping unit comprises a polypeptide monomer.

Claim 149 (new): The method of claim 146, wherein the therapeutic agent or drug encapsulating step is carried out by mixing the polymer and the therapeutic agent or drug molecule together in a solution such that the therapeutic agent or drug molecule can permeate inside the polymer.

Claim 150 (new): The method of claim 145, further comprising attaching a targeting molecule or a vector to the therapeutic agent- or drug-loaded polymer during the encapsulation process or after the completion of the encapsulation process.

Claim 151 (new): The method of claim 145, further comprising using lipids or lipid molecules during the encapsulation process.

Claim 152 (new): The method of claim 151, wherein liposomes are induced to form from the lipids in the presence of both the therapeutic agent or drug molecules and the monomeric polypeptides.

Applicant : Sh rt, et al.
Serial No. : 09/997,807
Filed : November 30, 2001
Page : 8 of 16

Attorney's Docket No.: 09010-910001

Claim 152 (new): The method of claim 31, further comprising attaching the polymer to a hydrogel.

Claim 153 (new): The method of claim 152, wherein the hydrogel comprises a three-dimensional structural network for a biochip.

Claim 154 (new): The method of claim 32, wherein the monomeric polypeptide has an amino acid sequence as set forth in SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, or SEQ ID NO:10.

Claim 155 (new): The method of claim 31, wherein the monomeric polypeptide has an amino acid sequence having at least 50% sequence identity to an amino acid sequence as set forth in SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, or SEQ ID NO:10 over at least about 40 consecutive amino acid residues.

Claim 156 (new): The method of claim 155, wherein the sequence identity is at least 55%.

Claim 157 (new): The method of claim 156, wherein the sequence identity is at least 60%.

Claim 158 (new): The method of claim 157, wherein the sequence identity is at least 65%.

Claim 159 (new): The method of claim 158, wherein the sequence identity is at least 70%.

Claim 160 (new): The method of claim 159, wherein the sequence identity is at least 75%.

Applicant : Short, et al.
Serial No. : 09/997,807
Filed : November 30, 2001
Page : 9 of 16

Attorney's Docket No.: 09010-910001

Claim 161 (new): The method of claim 160, wherein the sequence identity is at least 80%.

Claim 162 (new): The method of claim 161, wherein the sequence identity is at least 85%.

Claim 163 (new): The method of claim 162, wherein the sequence identity is at least 90%.

Claim 164 (new): The method of claim 163, wherein the sequence identity is at least 95%.

Claim 165 (new): The method of claim 164, wherein the sequence identity is at least 97%.

Claim 166 (new): The method of claim 155, wherein the sequence identity is over at least about 50 consecutive amino acid residues.

Claim 167 (new): The method of claim 166, wherein the sequence identity is over at least about 75 consecutive amino acid residues.

Claim 168 (new): The method of claim 167, wherein the sequence identity is over at least about 100 consecutive amino acid residues.

Claim 169 (new): The method of claim 168, wherein the sequence identity is over at least about 150 consecutive amino acid residues.

Claim 170 (new): The method of claim 169, wherein the sequence identity is over the full length of the polypeptide.

Applicant : Short, et al.
Serial N . : 09/997,807
Filed : November 30, 2001
Page : 10 of 16

Attorney's Docket No.: 09010-910001

Claim 171 (new): The method of claim 33, wherein the sequence identity is at least 55%.

Claim 172 (new): The method of claim 171, wherein the sequence identity is at least 60%.

Claim 173 (new): The method of claim 172, wherein the sequence identity is at least 65%.

Claim 174 (new): The method of claim 173, wherein the sequence identity is at least 70%.

Claim 175 (new): The method of claim 174, wherein the sequence identity is at least 75%.

Claim 176 (new): The method of claim 175, wherein the sequence identity is at least 80%.

Claim 177 (new): The method of claim 176, wherein the sequence identity is at least 85%.

Claim 178 (new): The method of claim 177, wherein the sequence identity is at least 90%.

Claim 179 (new): The method of claim 178, wherein the sequence identity is at least 95%.

Claim 180 (new): The method of claim 179, wherein the sequence identity is at least 97%.

Applicant : Short, et al
Serial No. : 09/997,807
Filed : November 30, 2001
Page : 11 of 16

Attorney's Docket N .: 09010-910001

Claim 181 (new): The method of claim 33, wherein the sequence identity is over at least about 150 consecutive residues.

Claim 182 (new): The method of claim 181, wherein the sequence identity is over at least about 200 consecutive residues.

Claim 183 (new): The method of claim 182, wherein the sequence identity is over at least about 300 consecutive residues.

Claim 184 (new): The method of claim 183, wherein the sequence identity is over at least about 400 consecutive residues.

Claim 185 (new): The method of claim 184, wherein the sequence identity is over the full length of the nucleic acid.

Claim 186 (new): The method of claim 31, wherein the polypeptide is encoded by a nucleic acid that hybridizes under stringent conditions to a nucleic acid having a sequence as set forth in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, or SEQ ID NO:9, wherein the stringent conditions comprise a washing step comprising 2X SSC, 0.1% SDS at room temperature for 15 minutes.

Claim 187 (new): The method of claim 31, wherein the polypeptide is encoded by a nucleic acid that hybridizes under stringent conditions to a nucleic acid having a sequence as set forth in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, or SEQ ID NO:9, wherein the stringent conditions comprise a washing step comprising 0.1X SSC, 0.5% SDS at room temperature for 30 minutes to 1 hour.

Claim 188 (new): The method of claim 31, wherein the polypeptide is encoded by a nucleic acid that hybridizes under stringent conditions to a nucleic acid having a sequence

Applicant : Short, et al.
Serial No. : 09/997,807
Filed : November 30, 2001
Page : 12 of 16

Attorney's Docket No.: 09010-910001

as set forth in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, or SEQ ID NO:9, wherein the stringent conditions comprise a washing step comprising 0.1X SSC, 0.5% SDS for 15 to 30 minutes at between the hybridization temperature and 68°C.

Claim 189 (new): The method of claim 32, wherein the conservative amino acid substitution comprises substituting one amino acid for another of the same class.

Claim 190 (new): The method of claim 189, wherein the conservative amino acid substitution comprises substitution of one hydrophobic amino acid for another, or, substitution of one polar amino acid for another.

Claim 191 (new): The method of claim 190, wherein the conservative amino acid substitution comprises substitution of isoleucine, valine, leucine or methionine, for another hydrophobic amino acid.

Claim 192 (new): The method of claim 190, wherein the conservative amino acid substitution comprises substitution of arginine for lysine, glutamic acid for aspartic acid or glutamine for asparagine.